

Clinical Benefit of Idiotypic Vaccines: Too Many Trials for a Clever Demonstration?

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Abstract: The most accepted and least biased manner to demonstrate clinical benefit for any new treatment is to show that it conveys a survival advantage in a well-designed phase III, randomized clinical trial. However, in selected cases, an exception can be made to this sound rule.

This review aims at elucidating one such example. In particular, I intend to show that when an individualized form of immunotherapy like idiotypic vaccination, which by definition is completely inactive against any tumor cells, is applied to cancer patients with indolent follicular lymphoma, a carefully crafted phase II clinical trial may be able to demonstrate clinical benefit better and more rapidly than a phase III alternative.

This consideration might be rather important over the next two to three years, since the results of as many as three ongoing phase III clinical trials on idiotype vaccines are expected to be unveiled within this time frame, following the release of conclusive data of our phase II clinical trial, which is imminent.

INTRODUCTION

To date, immunotherapy has done far less for cancer patients than cancer patients have done for immunotherapy, particularly in the field of tumor vaccine-induced clinical benefit.

While facing this reality, it seems appropriate to reiterate that not all clinically tested cancer vaccines are equal, nor are the actual results achieved so far through their use.

To date, most if not all cancer vaccines have shown at the most anecdotal successes in the clinical setting [1], with the exception of idiotype (Id) vaccines for B-cell malignancies, particularly follicular lymphoma (FL) [2]. A discussion of the list of possible reasons for this discrepancy falls outside the scopes of this review. However, it is the author's opinion that any such analysis should start from the crucial problem of vaccine antigen identification. The most likely reason for the greater effectiveness of Id vaccines likely depends on the intrinsic, structural and biological nature of the targeted antigens. The Id-containing immunoglobulin is a well-characterized and tumor-specific whole protein [3], not merely a group of unevenly-immunogenic, tumor-associated peptides like those typically used, either separately or in combination, as vaccines in several cancer types other than B-cell malignancies [1].

Yet, Id vaccines themselves have not culminated their development process, inasmuch as the clinical benefit obtained from their use remains to be formally proven [4].

The Idiotype

The term Id defines the collection of idiotopes. Idiotopes are a peculiar variety of antigenic determinants or epitopes exclusively found on the immunoglobulin's heavy and light chain variable regions [3]. Each immunoglobulin features its own Id and identical Ids define identical immunoglobulins. Therefore, the Id can serve as a B-cell malignancy-specific antigen for vaccine therapy, provided that the tumor cells express it on their cell membrane both/either intact as it is in its regular function as B-cell receptor and/or in the form of idiotopes associated with the HLA molecules for epitope presentation [5]. Then, in order for the vaccine-induced immune response to ultimately kill tumor cells *in vivo* [6], an efficient vaccine is expected to elicit both specific helper T-cells (T_h) and an Id-specific humoral response [4,6], which might trigger in turn natural killer cells (NK) for an antibody-dependent, cell-mediated cytotoxicity (ADCC) [4]. Another mechanism by which a vaccine may kill tumor cells is by eliciting a specific T-cell response, including cytotoxic T lymphocytes (CTLs) [6].

Of course, the ultimate biological goal of idiotypic vaccination is to elicit and maintain in the long term both humoral and cellular immune responses. However, at least in some patients it may be easier to achieve that goal than to be able to demonstrate it in the lab due to the lack of clear cut, easily reproducible tests, particularly for T-cell response detection and monitoring. We measure *in vivo* immune function poorly in people. Nevertheless, the formal proof of a vaccine-induced clinical benefit has remained elusive so far. Moreover, should such evidence emerge, it would remain to be defined what the achievable goal of Id vaccines should be, that is either tumor eradication or control. In fact, in logistical terms the former aim might be likely pursued

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through a relatively short vaccination schedule and therefore with a limited number of vaccine doses, whereas the latter would imply the need for long term boost production and administration.

Soluble Protein Idiotype Vaccines

The most data on Id vaccines have been generated using the whole tumor-specific immunoglobulin in the form of a soluble protein conjugated to the immunological carrier keyhole limpet hemocyanin (KLH) and added to granulocyte-macrophage colony-stimulating factor (GM-CSF), which functions as an immunological adjuvant [7].

This vaccine formulation, which is currently used in all four clinical trials that constitute the object of the present review, is the result of preliminary and yet sound conclusions drawn from two seminal clinical studies.

The original clinical trial conducted at Stanford University [8] has been expanded over the last fifteen years in terms of both number of FL patients enrolled [4,9] and vaccine formulation refinement [4,9]. Some of its findings are already conclusive and unchallenged, whereas others remain either hypothetical or to be confirmed. In general, the conclusions can be outlined as follows: it is possible to immunize a cancer patient against an antigen solely expressed by his/her own tumor [8]; Id vaccine can elicit Id-specific humoral responses, also implying the activation of specific T_h cells [8]; it is plausible that Id-specific humoral responses may trigger NK-mediated ADCC against Id-bearing tumor cells [4]; the survival curve trends favor the hypothesis that patients in whom the Id vaccine succeeds at eliciting a specific immune response, particularly a humoral response, fare better than those who do not [4,9]. Still, all these studies logically lacked any formal and comparative control patient population, so that the last conclusion is based on a form of analysis that has been discredited. Patients who fail to make a response may be sicker; thus, the fact that they did not fare as well cannot be said to reflect a positive treatment effect in those who responded.

A second important clinical trial, conducted at the National Cancer Institute [6], has been partially updated recently [10]. Its conclusions can be summarized as follows: the addition of GM-CSF to the vaccine formulation appears to be able to improve both frequency and quality of Id- and tumor-specific T-cell responses, including the induction of specific CTLs, without reducing Id-specific B-cell responses [6]; vaccine-induced T- and/or B-cell responses are capable of killing tumor cells *in vivo* that had survived pre-vaccine chemotherapy [6]; most patients experiencing vaccine-induced, Id-specific immune responses tend to maintain them at least six months after stopping active vaccination [6]; still, an important fraction of these patients will eventually both lose that immunity and relapse [10], suggesting but not proving that most patients might benefit from long term booster vaccine administration.

Finally, both trials have shown that soluble protein Id vaccines are both nearly non-toxic and substantially harmless to FL patients [4,6].

Follicular Lymphoma and Phase III Clinical Trials

Currently, FL falls into the group of diseases least suitable for conducting phase III clinical trials. This assessment is based on several factors.

First, FL is a generally indolent variety of cancer and such a feature, which is mostly to the patient's advantage, represents a double-edged sword for scientists involved in clinical investigation. Indeed, the prolonged overall survival (OS) and the relative lack of impact on it of treatment make most such trials non viable because of the enormous amount of time necessary to detect survival differences, if such differences exist.

Moreover, the fact that the disease is not curable, but also yields a relatively good life expectancy, makes patients less prone to conform with the rigid norms of randomized clinical trials, particularly when a patient is assigned to the control arm and other new drugs become readily available.

Furthermore, FL is well known for its variable and unpredictable natural history, not only when we compare different cases apparently featuring very similar clinical characteristics, but also sometimes even when the analysis is limited to the clinical history of individual cases. This fact makes general comparisons less straightforward than in other clinical cancer settings.

Finally, no substantial consensus exists about what the concept of standard therapy means in FL treatment and, therefore, what clinically and ethically sound control arm should be used.

Several new agents have recently reached the clinical use stage and obtained full acceptance for the treatment of FL patients. Probably none of these drugs is capable of curing the disease, either alone or in combination, but each appears to be able to provide clinical benefit. Consequently, this situation inevitably generates both fierce commercial competition and unwillingness to conduct comparative clinical studies capable of generating conclusive results about the real efficacy of each treatment option. For instance, plenty of resources have been spent to formally demonstrate that the R-CVP chemotherapy regimen is more effective than CVP alone [11], as if there had ever been any biological or clinical reason to think otherwise. On the contrary, it is proving to be much more difficult to test whether sequential treatment including anti-CD20 both cold and radionuclide-conjugated [12,13] monoclonal antibodies may or may not compare favorably with standard chemotherapy regimens with or without the addition of an autologous stem cell transplantation. These considerations also hold true for custom-made, patient-specific therapies such as Id vaccines. Nonetheless, just the opposite has occurred. Despite dealing with both the same type of treatment and the same FL patient population, as many as three independent and competing phase III clinical trials have been launched almost contemporaneously and exclusively for commercial reasons. Moreover, depending on their features, it can be demonstrated that none of them has been designed to answer all the questions that such phase III clinical trials are supposed to answer.

However, aside from discussing all these features, the primary aim of this review is to show that Id vaccine clinical benefit, if any at all, can be demonstrated both more effectively and rapidly through a carefully-crafted phase II than by a conventional phase III clinical trial.

Basic Concepts Concerning Phase III and II Clinical Trials

The two fundamental concepts required to understand the meaningfulness of a phase III clinical trial are the following: first, at least in the experimental arm, each patient must receive a treatment supposedly active against his/her disease; second, within each arm each patient is meant to receive exactly the same treatment as everybody else. Of course, within each arm different patients may receive different total doses of the same drugs based on several factors detailed in the protocol, such as their different weight, body surface area, drug clearance capacity, etc. However, given the ultimate goal of comparing new agents with the standard treatment, the crucial methodological tool is to provide each patient with both the same drugs and the same dose intensity as everybody else enrolled in the same study arm. Almost all phase III clinical trials fulfill both requirements. Ongoing phase III clinical trials on Id vaccine treatment for newly diagnosed FL patients do not.

In fact, all such trials feature a post-chemotherapy randomization between patients receiving the complete Id-vaccine and those who receive only its non-specific components, that is KLH and GM-CSF. This means that the latter are treated with two compounds for which no activity against FL has ever been demonstrated, whereas the former are treated with three compounds that cannot kill FL cells. The Id itself is harmless to the tumor cell by definition, being a FL-specific molecule. More importantly however, each patient within the experimental arm receives his/her own FL-specific Id, which is intrinsically different from that of each and every other patient, particularly as far as its immunogenicity is concerned [14-17]. Paradoxically, the core of the whole experimental treatment has neither of the requirements for a phase III study: it is not directly active against the tumor and it is never the same one patient after another.

This latter problem generates the oxymoronic situation of randomized studies in which the sole patient population to be uniformly treated is that corresponding to the control arm, whereas the experimental arm gathers together patients immunized with vaccines that by their very nature possess an extremely variable degree of both potential immunogenicity and efficacy. This fact casts gigantic shadows on the chances of such studies for success.

Of course, there is no such a thing as the perfect phase III clinical trial. There are always individual patient-related variables whose influence may be so unpredictable that they simply cannot be taken into account at the time of study design: for instance the impact of multi-drug resistance in a study on a chemotherapy agent/regimen or the relationship between graft-versus-host and graft-versus-disease in a study on allogeneic stem cell transplantation. However, in all these cases it is at least known that, in principle, the experimental treatment option has a theoretical chance to directly inflict

damage to the tumor cells. In the case of Id vaccines, and even without taking into account the post-chemotherapy functional status of each patient's immune system, all Ids are *per se* inactive against the tumors from which they have been derived, while each patient's Id is differently immunogenic from that of any other patient enrolled in the same study arm. In a sense, all these phase III clinical trials are comparing what happens to hundreds of patients not vaccinated against their own tumor, and considered as if they were a uniform patient population despite the great degree of biological and clinical variability of FL, to what happens to hundreds of patients vaccinated against FL, each of them in a substantially different manner.

Shifting to phase II clinical trials, the main reason to explain why most of them are unable to convincingly prove clinical benefit is that almost invariably they lack an adequate control patient population. Even when the patients under experimental treatment are matched to an historical control, the meaningfulness of this comparison is inevitably small due to a variety of both obvious and well-known biases.

Taking into account all considerations above, we decided to carefully design a peculiar phase II trial on Id vaccines as a treatment for FL patients in first relapse. In particular, each patient enrolled in it systematically serves as both the case and its intrinsically matched control. Intuitively then, when using each patient's data, we are employing them also as an excellent control in both clinical and biological rather than solely historical terms.

Accentia Biopharmaceuticals-Sponsored Clinical Trial (BIOVAXID)

This phase III clinical trial was launched at the National Cancer Institute five years ago and unfortunately has been characterized by an unusually slow accrual pace ever since [18]. This fact might represent the most serious reason of possible failure of the whole study in the long term [7]. However, it has to be credited with the highest degree of intellectual integrity, as it represents the sole randomized study on Id vaccines generated as a consequence of clear cut results obtained in previous phase I and II clinical trials. In fact, even the custom-made vaccine production strategy has not been changed when shifting from small to large size studies in terms of number of patients enrolled and this fact guarantees a substantial scientific continuity while moving from biological to clinical efficacy demonstration. In particular, the Id is obtained through the original rescue fusion method [19,20] previously utilized to demonstrate all major proofs of principle of Id-based vaccines [6,8,9]. As opposed to the other randomized trials, this then is the sole study that can claim full and formal adherence to common scientific criteria, according to which scientific findings should reach clinical application after carefully evolving from pre-clinical through translational research.

Other points of strength of the trial are represented by the systematic PCR assessment of minimal residual disease [6], for more than just clinical comparisons between the two arms of the study, and by the decision of administering either the Id vaccine or the placebo only to patients achieving a clinical complete response (CR) following pre-vaccine

chemotherapy. However, precisely the choice of the study pre-vaccine chemotherapy raises at least some perplexities. The PACE (Cyclophosphamide, Doxorubicin, Etoposide and Prednisone) regimen is not widely used for FL treatment and it was chosen because it was supposedly more effective than other standard regimens such as CHOP and CVP in terms of number and duration of the CRs that it may induce. The problem with this choice is that, assuming this higher efficacy presumption is correct, such a regimen might delay the demonstration of an Id vaccine-related, clinical advantage, that is disease-free survival (DFS) and OS, in the experimental arm. This issue might further burden a trial already struggling with a conspicuous enrollment delay.

Moreover, as it becomes more and more evident that the addition of rituximab to nearly any chemotherapy regimen tends to improve at the very least the response rates in FL patients, the absence of this monoclonal antibody in the pre-vaccine treatment schema will inevitably make enrollment all the more difficult, possibly even from an ethical standpoint.

Finally, due to its design (Fig. 1), this study will not be able to investigate the eventual impact of Id vaccine boosts in order to maintain the benefit, if any, from the standard short course of vaccinations.

Genitope Corporation-Sponsored Clinical Trial (MyVax)

Contrary to the previous study, despite having being launched later this trial has concluded the patient enrollment phase in a timely fashion. Its planned final result report is due in 2007, but the content of its first interim analysis has been released recently. In particular, no statistical differences in progression-free survival (PFS) have yet been found comparing vaccinated patients to those in the control arm.

Mainly for logistical reasons, the vaccine production has been based on recombinant rather than traditional rescue fusion technology and the implications of this dramatic methodological change are still far from having being elucidated [7]. In particular, it is unknown whether all proofs of principle above may still hold true as a recombinant Id obtained through mammalian cell transfection is used to

vaccinate patients instead of that rescued from hybridoma supernatants. Another important feature of this study is that patients achieving either a clinical CR or a partial response (PR) are vaccinated. This fact will generate as many as four rather than two groups of treated patients to be compared, so that the number of evaluable patients in each group might limit the likelihood of obtaining statistical significance in PFS differences. In this respect, the choice of pre-vaccine chemotherapy might come to the aid of the investigators. Indeed, the CVP regimen alone is quite unlikely to achieve long lasting responses [11], so that in the hypothesis that the Id vaccine be clinically beneficial, it should provide a clear cut PFS advantage at the very least to patients vaccinated in first clinical CR. However, there has been an important price to be paid for this chemotherapy choice: that of knowingly undertreating most if not all patients enrolled in the control arm of the trial. In fact, over the last four years CVP alone has ceased to be a standard chemotherapy regimen for FL patients needing treatment in most developed countries [21]. Notably and probably related once again to the choice of CVP, no minimal residual disease-related endpoint is considered among the primary objectives of this study, although it is plausible that such important data may be retrospectively analyzed.

Finally, Id vaccine boost administration is not part of this trial either, and in the long term this fact might become a point of weakness of the whole study design (Fig. 2).

Faville-Sponsored Clinical Trial (FavId)

The third and last phase III trial launched to demonstrate clinical benefit of idiotypic vaccination in a randomized setting is seemingly encountering no problems in terms of patient accrual. What instead calls most attention to this study is the conspicuous number of unconfirmed hypotheses that this trial has been built upon [22].

The method chosen to rescue the tumor-specific, custom-made Id is once again based on the recombinant technology, with all implications mentioned while describing the previous study's main features. Additionally, it has to be noted that the transfectants are insect rather than mammalian

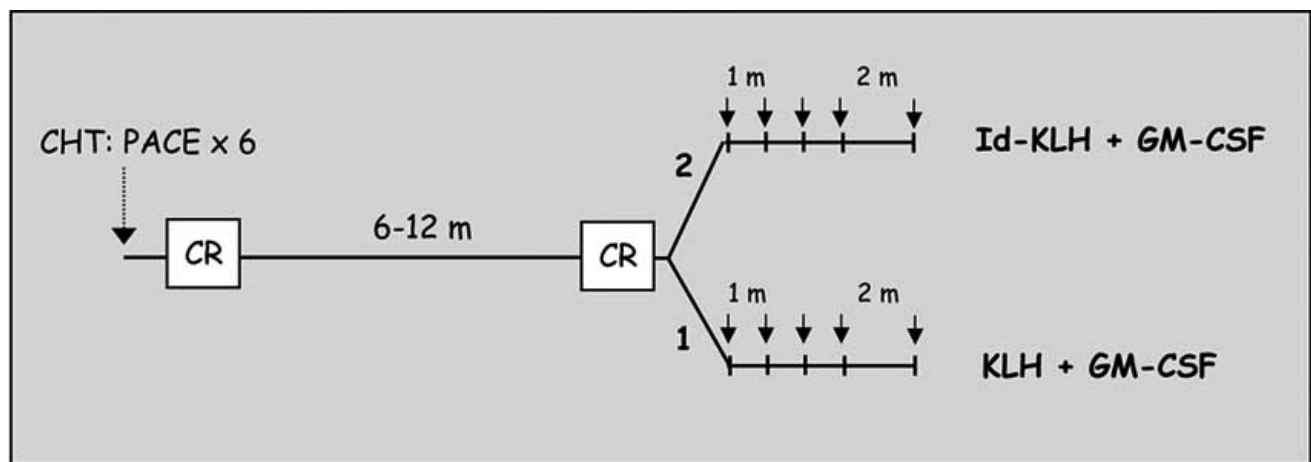


Fig. (1).

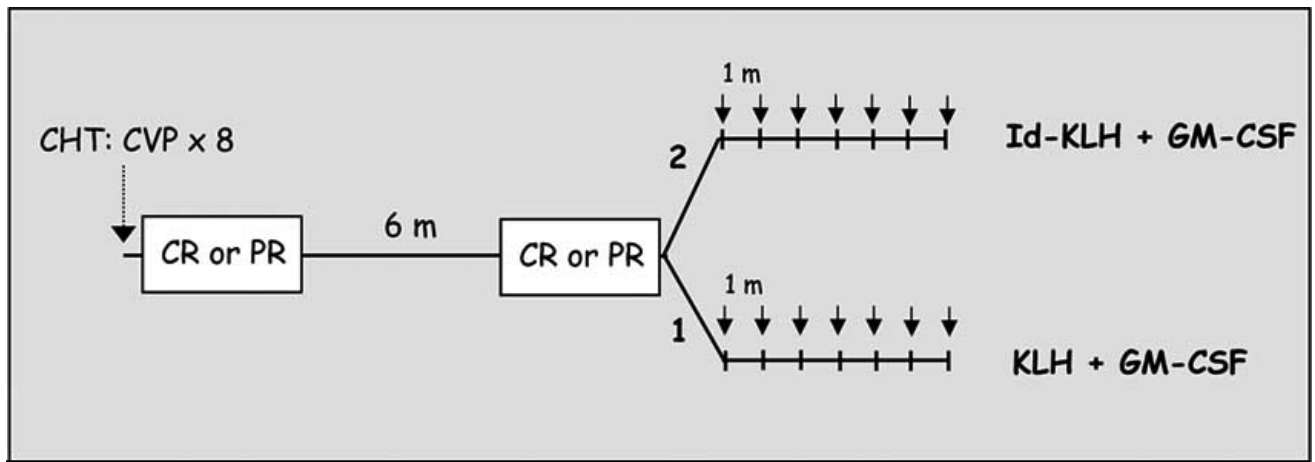


Fig. (2).

cells. Of course, this fact raises even greater concerns about the overall immunogenicity of the Id protein, particularly as far as the unknown effects of different patterns of Id glycosylation [22-24].

In terms of study design (Fig. 3), this randomized trial differs substantially from the others. First, FL patients are enrolled without considering their histological grade [25]. This fact is at odds with current clinical practice, according to which grade IIIb FL should neither be considered as an indolent lymphoma, nor be treated as such [26]. On the contrary, like any other FL case enrolled in this trial, patients with grade IIIb FL must undergo single agent rituximab treatment before being randomized to receive either vaccination or placebo. Second, this study is based on a 1:1 rather than 2:1 randomization. This fact, together with the clinical characteristics of the patients enrolled, once again might limit the chances of rapidly demonstrating statistical differences in terms of PFS. As a matter of fact, no other Id vaccine trial presumes to enroll such a non-homogeneous FL patient population, including newly diagnosed cases, those that have never been treated before and those relapsed with no more than two previous treatments; nor does any other Id

vaccine trial assign to either arm patients who have achieved even just a disease stabilization (SD) following rituximab treatment.

As for the pre-vaccine therapy, possible concerns are similar to those already mentioned for CVP alone. In particular, four weekly administrations of single agent rituximab are widely accepted as not being the best standard option for FL patients who need treatment. In this respect, once again it does not come as a surprise that this study also does not include minimal residual disease monitoring among its main endpoints. Moreover, it is generally considered that the prolonged, rituximab-induced B-cell depletion is mechanistically at odds with the desirable early induction of Id-specific humoral responses through idiotypic vaccination [4,27]. In this respect, it does not seem convincing at all that, before an experimental treatment such as Id vaccine, another still relatively new drug be used as single agent, solely based on an unconfirmed pre-clinical report [22,28].

On the contrary though, this is the only randomized trial on Id vaccines aiming at elucidating the possible clinical impact of boost vaccine administration.

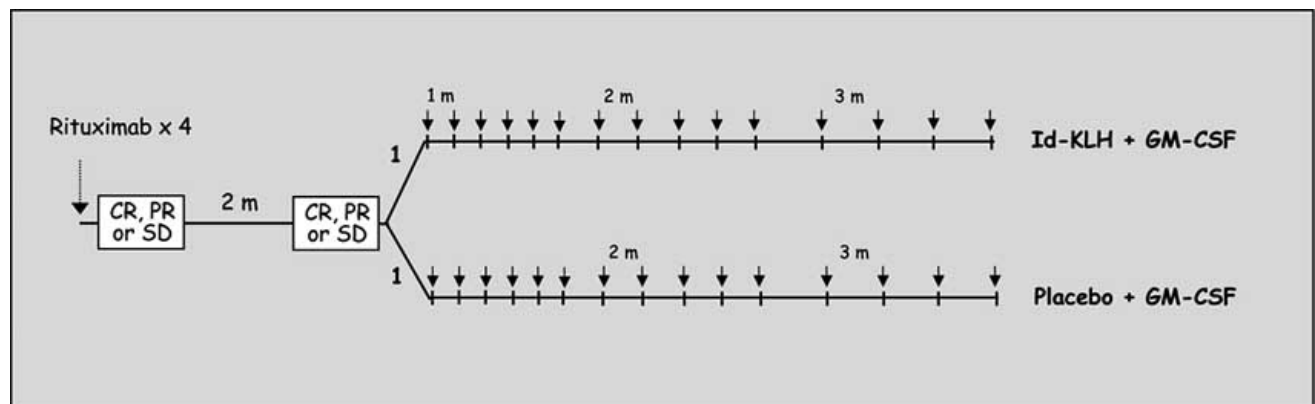


Fig. (3).

University Clinic of Navarre (CUN)-Sponsored Clinical Trial (FLIDVAX)

This is an unusual phase II clinical trial, whose design (Fig. 4) is strictly based on three clinical dogmas applying to FL [7]. First, that conventional treatment-induced second clinical CR will be shorter than first clinical CR in most if not all patients [29,30]. Second, that the median duration of a second clinical CR achieved through conventional treatment, including the CHOP regimen, is about 13 months [30,31]. Third, that if the Id vaccine is in principle unable to provide any clinical benefit, it is impossible that any FL patient in CHOP-induced second clinical CR will not relapse within 26 months, despite being actively vaccinated and showing a vaccine-induced, Id-specific immune response throughout the same time frame [7]. The accrual was completed in June 2004 and the release of the striking final results is imminent.

Contrary to the phase III studies above, this trial solely enrolls FL patients in first relapse, most of whom had already received rituximab and/or fludarabine as first line agents and therefore could be treated at the time of relapse with CHOP alone, which is similarly effective [32] but less demanding in terms of rapidity of post-chemotherapy B- and T-cell recovery.

As in the case of the first phase III study ever launched, the Id production is based on the traditional rescue fusion method [19,20], which guarantees the use of a vaccine that has already proven biologically effective *in vivo* [4,6]. Moreover, the postulated clinical benefit of a vaccine-induced immune response is being also investigated in terms of possible impact on minimal residual disease [6]. Remarkably though, this is the sole trial in which the duration of the off-therapy period between pre-vaccine chemotherapy completion and vaccination start is not pre-set, but rather depends on documented full numerical recovery of circulating CD3+, CD4+, CD8+ and CD19+ lymphocyte subpopulations.

Finally, as mentioned this study also explores the role of vaccine boosts throughout a period of time twice as long as the median duration of a CHOP-induced second clinical CR in FL.

CONCLUSION

Among the four studies, which represent the subject of this review, only one was purposely designed not to be randomized: ours. It was decided to conduct it this way mainly because we strongly defend that, in order to demonstrate clinical benefit of a completely custom-made treatment such as idiotypic vaccination in FL, the concept of randomization is greatly misleading. Therefore, it does not come as a surprise to us seeing how none of the three competing and contemporary phase III, randomized clinical trials conceived with the same goal ended up with a study design sufficiently satisfactory.

In terms of accrual, only the Accentia-sponsored trial is continuing to experience serious problems. From a methodological standpoint, both the Genitope- and the Favrilite-sponsored studies are being conducted as if recombinant Id vaccines might already guarantee at least the same degree of biological efficacy as those obtained through rescue fusions, but this hypothesis is unproven.

As far as the study design is concerned, virtually no trial can claim being flawless for a number of reasons detailed above, that is likely unworthy to stress further. Finally, only two studies are focusing on the role of Id vaccine boosts at least in those patients who achieve a vaccine-induced, Id- and/or tumor-specific immunity.

Future Developments

Despite the inevitable struggle that the demonstration of clinical benefit of a custom made immunotherapy procedure implies, it is the author's opinion that Id vaccines will prove clinically beneficial to FL patients sooner than might be expected. Should this be the case, it is also the author's opinion that the whole therapeutic flow chart for FL treatment might soon include the combination of rituximab and fludarabine, in association or not with more conventional agents, as first line therapy followed by Id vaccine starting around one year thereafter. Contemporarily, first relapse FL patients might then receive radioimmunotherapy followed or not by autologous stem cell transplantation and by idiotypic vaccination, particularly if either were never received as first line treatment or were given with no response the first time.

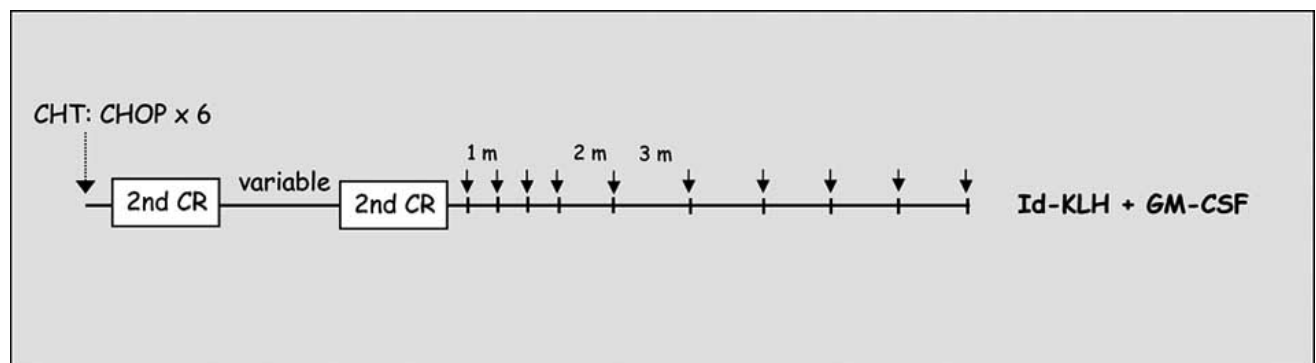


Fig. (4).

ABBREVIATIONS**General**

FL	=	Follicular lymphoma
HLA	=	Human leukocyte antigen
PCR	=	Polymerase chain reaction

Standard Treatment

R	=	Rituximab
CVP	=	Cyclophosphamide, Vincristine, Prednisone
CHOP	=	Cyclophosphamide, Doxorubicine, Vincristine, Prednisone
PACE	=	Prednisone, Doxorubicine, Cyclophosphamide, Etoposide

Response to Treatment

CR	=	Complete response
PR	=	Partial response
SD	=	Stable disease
OS	=	Overall survival
DFS	=	Disease-free survival
PFS	=	Progression-free survival

Immunotherapy

Id	=	Idiotype
KLH	=	Keyhole limpet hemocyanin
GM-CSF	=	Granulocyte-monocyte colony-stimulating factor
Th	=	Helper T cell
CTL	=	Cytotoxic T lymphocyte
NK	=	Natural killer
ADCC	=	Antibody-dependent cell-mediated cytotoxicity

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